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THE DGAT-1 GENE POLYMORPHISM IS INFORMATIVE QTL MARKER FOR MEAT QUALITY IN BEEF CATTLE

Piotr Urtnowski, Jolanta Oprzadek, Adrianna Pawlik, Edward Dymnicki

Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzebiec, 05-552 Wolka Kosowska, Poland
p.urtnowski@ighz.pl

Acyl-CoA:diacylglycerol acyltransferase-1 (*DGAT-1*) is a key enzyme involved in triglyceride synthesis. *DGAT-1* gene is located in the centromeric region of the bovine chromosome (BTA) 14 and considered polymorphism was identified as a candidate gene for milk and meat QTL. One of mutations which is substitution AA → GC in exon 8 causes an amino acid change in the product. The effect of the lysine/alanine (K232A) diallelic polymorphism on meat production traits has been studied. 156 young Black-and-White (Friesian) bulls were genotyped. The association between diacylglycerol acyltransferase polymorphism and slaughter performance and meat technology analysis were examined. Moreover the fatty acid profile (C12–C20:5) including CLA in the sample of *longissimus dorsi* (LD) muscle was evaluated. Differences ($P \leq 0,05$) were found between genotypes in slaughtering performance traits as well as fat and valuable cuts content and meat-fat ratio. The significant difference occurred also in the water holding capacity. Heterozygous individuals AA:GC were characterized by the greatest values compared with homozygous ones. Bulls of *DGAT-1* genotype GC:GC showed significantly higher ($P \leq 0,05$) content of lauric acid in LD muscle and heterozygous animals differ in CLA content with homozygous AA:AA.

Key words: *DGAT-1*, cattle, beef, gene polymorphism, carcass traits

ПОЛИМОРФИЗАМ НА ГЕНОТ DGAT-1 Е ИНФОРМАТИВЕН QTL МАРКЕР ЗА КВАЛИТЕТ НА МЕСО ПРИ ОДГЛЕДУВАЊЕТО НА ГОЈНИ ГОВЕДА

Ацил-CoA:диацилглицерол-ацилтрансфераза-1 (DGAT-1) е клучен ензим вклучен во синтезата на триглицеридите. Генот на DGAT-1 е лоциран во регионот на центромерите во бовинскиот хромозом (BTA) 14 и анализираниот полиморфизам беше идентификуван како ген-кандидат за млеко и месо QTL. Една од мутациите, која претставува супституција AA→GC во егзон 8, предизвикува аминокиселинска промена во производот. Беше проучуван ефектот на диалеличен полиморфизам на лизин/аланин (K232A) врз својствата на месопроизводството. Беше одреден генотипот кај 156 млади црно-бели (фризиски) бикови. Проучувана е врската помеѓу полиморфизмот на диацилглицерол-ацилтрансфераза и изведбата на колењето на добиток и анализата на производство на месо. Покрај тоа, оценуван е профилот на масна киселина (C12–C2 0:5) вклучувајќи CLA во примерок од мускулот *longissimus dorsi* (LD). Беа утврдени разлики ($P \leq 0,05$) помеѓу генотиповите во однос на кланичните перформанси на добитокот, содржината на маст, отпадните делови и соодносот месо–маст. Беше утврдена значителна разлика во однос на способноста за задржување на вода. Повисоки вредности беа утврдени кај хетерозиготните индивидуи AA:GC, во споредба со хомозиготните. Биковите со генот DGAT-1 GC:GC покажаа значително повисоко ниво ($P \leq 0,05$) на лауринска киселина во мускулот LD и хетерозиготните животни се разликуваат во присутноста на CLA од хомозиготните AA:AA.

Клучни зборови: DGAT-1; говеда; телешко месо; генски полиморфизам; карактеристики на труп

1. INTRODUCTION

Fat content in carcass as marbling mainly, improves tenderness, flavour, colour and juiciness. Separated fatty acids in diet have strong influence on human health. Bovine fat is typically rich in saturated fatty acid, particularly palmitic acid, which is considered a hypercholesterolemic factor whereas certain unsaturated fatty acids are stated

to show antiatherogenic or even anticarcinogenic properties in human (Scheeder et al. 2001). Thus, fat content and fatty acid profile are also very important for both, consumers and producers. To make the selection for economically important traits more efficient, Marker Assisted Selection (MAS) has been established. However, the efficient use of *QTL* (Quantitative Trait Loci) information in selective breeding requires precise mapping and knowledge about the molecular basis of the *QTL* variation. The identification of *QTL* with influence on production traits in cattle breeds has been objective of many studies (Moore et al. 2003; Casas et al. 2005; Sanders et al. 2006). The diacylglycerol *O*-acyltransferase (DGAT1) is a microsomal enzyme that catalyzes the final step of the triglyceride synthesis. Acyl-CoA:diacylglycerol acyltransferase-1 (*DGAT-1*) gene is located on the chromosome 14 which has been found as a region rich in milk and meat *QTL* (Grisart et al. 2002; Winter et al. 2002). The recent work has evidenced a significant association between lysine at amino acid position 232 with elevated milk fat content, while an alanine at this position is associated with lowered milk fat content. Sequencing of *DGAT-1* from pooled DNA revealed significant frequency shift at several variable positions between groups of animals with high and low breeding values for milk fat content in different breeds. Among the variants was a substitution of lysine by alanine (K232A), with lysine-encoding allele being associated with higher milk fat content. Haplotype analysis indicated the lysine variant to be ancestral (Winter et al. 2002; Kaupe et al. 2004).

Triglyceride (TG) is the major energy storage form and is synthesized primary in liver, adipose tissue and small intestine (Lehner, Kuksis, 1996). Acyl-CoA:diacylglycerol acyltransferase-1(*DGAT-1*) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis (Cases et al. 2001). Studies carried out on mice indicated that animals lacking both copies are completely devoid of milk secretion because of TG deficiency in the mammary gland (Smith et al. 2000). Moreover, animals have reduced adiposity and are resistance to diet-induced obesity through a mechanism that involves increased energy expenditure, what results from increased peripheral leptin sensitivity (Yamazaki et al. 2005). In this study an association was investigated between the polymorphism at *DGAT-1* and traits related to the meat production and quality in growing Polish Holstein young bulls.

2. MATERIAL AND METHODS

Animals. One hundred fifty six young Polish Holstein bulls were used from an experimental herd of the Institute of Genetics and Animal Breeding in Jastrzebiec, Poland. The animals were kept indoors and fed *ad libitum* Total Mixed Ratio (TMR) according to the INRA norm. The bulls were slaughtered at the age of 12 after 24 hours fasting. The carcasses were chilled for 24 hour at 4°C. Approximately 24 hours post-mortem samples of *Longissimus dorsi* (LD) muscle between the 11^{th} and 13^{th} rib were obtained from the right side of each animals for meat quality analysis and muscle lipid content and stored at –20˚C. Right carcass side was dissected. The estimation of the slaughter value was based on cold carcass weight, dressing percentage, weight of valuable cuts in carcass-side (round, shoulder, best ribs, fore ribs sirloin).

DNA isolation from the whole blood. An authorized veterinarian collected blood for isolation of DNA from the jugular vein. Blood was collected on K_2-EDTA and stored at –25°C for a few weeks or at –75°C up to several months. The isolation of the DNA from the whole blood was done with a rapid method described by Kanai et al. (1994).

DGAT-1 genotyping. The GC:AA polymorphism in exon 8 of the bovine DGAT-1 gene was identified using RFLP-*Cfr*I as described by Winter et al. (2002). The following primers were used to amplify a 411-bp DNA fragment encompassing parts of intron 7 and exon 8 of the *DGAT-1* gene: F – 5'-TCAGGATCCAGAGGTACCCAG-3' and R – 5'-GGGGTCCAAGGTTGATACAG-3'. The polymerase chain reactions were performed using a PCR-mix with both primers, each at a final concentration of 2 pmol/µl, 1 U Taq polymerase (Sigma), 1 µl Taq polymerase buffer, dNTPs of 2.0 mM/µl, ca 100 ng of genomic DNA, and H_2O up to 10 µl. The following PCR protocol was used 1 min at 94ºC, 1 min at 61ºC, and 1 min at 72ºC – 34 cycles. The yield and specificity of the PCR reactions were evaluated by electrophoresis of the products in 2% agarose gels (Gibco) with ethidium bromide.

The PCR products were digested in 10-µl aliquots with 10 U of *Cfr*I restriction nuclease (Bio-Labs, New England, USA) for 3 hours at 37ºC. The restriction fragments were subjected to elec-

trophoresis in 2% agarose/ethidium bromide gels (Gibco, BRL, England) in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). The gels were examined under UV light and documented in a FX Phosphoimager apparatus (Bio-Rad).

Chemical composition and physiochemical and sensory properties of meat. A part of each meat sample was used for taste-panel evaluation. The other part was mixed and used to determine the basic chemical composition and physiological properties. The sensory properties were evaluated according to 5-point scale (1 point – the lowest score to 5 point – the highest score). The taste panel evaluation was made by 5–person team characterized by higher than average sensory sensitivity. An analysis of the basic chemical composition of meat included the determination of the percentage water, protein, ash by conventional methods, pH (CP-315) measurement and water holding capacity.

Determination of muscle lipid content. To obtain a fatty acid profile from LD muscle the Soxhlet method had been used. From the extracts of isolated acids, through the hydrolysis, the derivatization had been carried around *p*-dibromo acetophenol and triethanolamine. Esters of fatty acids were analyzed (HPLC) on the column of the C-18 RP type. The concentration of fatty acids was read out based on the field of files. Standards of fatty acids came from the company SIGMA-ALDRICH.

Statistical model. Data were analyzed using the general linear model (GLM), procedure of SAS (SAS Inst. INC., Curry, NC.). We used one-way analysis of variance (ANOVA) to analyze differences among the groups. Post hoc comparisons were made using the Duncan's test. Comparisons with $P \leq 0.05$ were considered significant.

3. RESULTS AND DISCUSSION

The average weight of slaughtered animals was about 355 kg. The dressing percentage was low (51.64 %) because of the young age of animals. Valuable cuts constituted almost 61 %, in addition the percentage of meat in valuable cuts was high and presented the level over 70 % (Tab. 1).

The high water content in meat (76.47 %) and the low content of the fat (0.94 %) suggested incomplete slaughter maturity of animals. On the other hand, high protein percentage (over 21%) and high notes within the organoleptic evaluation indicate the high nutritional value (Tab. 2). Meat pH is one of the main characteristics of the technological usefulness of traits. At the normally glycolysis process the value after the end of 36 hours from slaughter is an average from 5.4 to 5.8. In the examined group pH was 5.53, what means, that animals weren't stressed out as well as they were in the good condition before slaughter. The amount of the cooking losses depends above all on the content of sarcoplazmatic proteins, the pH and water holding capacity (Tab. 2). The average amount of the cooking losses in presented samples was (7.43 %).

Amongst fatty acids these of the medium chain constituted the highest share (Tab. 7). The most characteristic acids for beef: palmitic and stearic and oleinic much exceeded the rest (Tab. 3).

When population is in a genetic balance, the observed proportion of genotypes is analogous to the proportion based on the frequency of alleles. Deviations can reveal the effect of the natural selection, the nonrandom mating system, migration from other population or structure of population. Observed numbers and frequency of genotypes and alleles were in Hardy-Weinberg equilibrium.

Table 1

Overall means and their standard deviation of analyzed traits in Polish Holstein bulls

Carcass traits	$\bar{x}$	SD
1. Average weight, kg	354.93	47.13
2. Cold carcass, kg	183.21	29.03
3. Dressing percentage, kg	51.64	4.04
4. Valuable cuts of carcass-side, kg	55.48	9.00
5. Lean weight of valuable cuts, kg	39.12	7.35
6. Bone weight of valuable cuts, kg	10.79	1.30
7. Fat weight of valuable cuts, kg	5.61	1.41
8. Valuable cuts share in carcass-side, %	60.79	1.41
9. Lean share of valuable cuts, %	70.45	3.61
10. Bone share of valuable cuts, %	19.65	1.74
11. Fat share of valuable cuts, %	10.15	2.01
12. Meat bones ratio	3.62	0.52
13. Meat fat ratio	7.31	2.08
14. Meat bone:fat ratio	2.41	0.43

Table 2

Overall means for meat quality features and their standard deviation in analyzed material

	Trait	$\bar{x}$	SD
	Physicochemical and technological parameters		
1.	Water content, %	76.47	0.94
2.	Protein content, %	21.14	0.78
3.	Fat content, %	0.94	0.44
4.	Total content of hem pigment [ppm hemin]	142.2	31.26
5.	pH	5.53	0.11
6.	Water holding capacity, cm^2/g	28.94	5.16
7.	Cooking losses, %	7.43	5.3
	Sensory properties		
1.	Colour, pt	4.58	0.38
2.	Taste, pt	4.51	0.37
3.	Flavor, pt	4.51	0.31
4.	Tenderness, pt	4.19	0.56

Table 3

Fatty acid composition and their standard deviation for Longissimus dorsi muscle

	Trait	$\bar{x}$	SD
	Fatty acid		
1.	C12	0.02	0.02
2.	C12_1	0.01	0.01
3.	C14	0.05	0.03
4.	C14_1	0.03	0.03
5.	C16	1.7	0.58
6.	C16_1	0.16	0.08
7.	C17	0.06	0.03
8.	C18	1.28	0.37
9.	C18_1C	2.24	0.91
10.	C18_1T	0.2	0.15
11.	C18_2	0.44	0.17
12.	C18_3A	0.04	0.02
13.	C20_3	0.1	0.04
14.	C20_4	0.17	0.07
15.	C20_5	0.01	0.02
16.	CLA	0.08	0.06

Two alleles (AA and GC) and three genotypes were identified. The AA/GC genotypes of the *DGAT-1* gene were estimated in the group of 156 animals bulls of Polish Holstein cattle. The frequency of AA:AA, AA:GC, and GC:GC genotype was 0.20, 0.47 and 0.33 respectively. The AA and GC alleles were represented with a frequency of 0.44 and 0.56 respectively (Tab. 4).

Table 4

Frequencies of DGAT-1 alleles and genotypes identified by the PCR-RFLP analysis

DGAT-1genotype frequency			Allele frequency	
AA:AA	AA:GC	GC:GC	AA	GC
0.20	0.47	0.33	0.44	0.56

Regarding valuable cuts of carcass-side (%) AA:AA animals exhibited the smallest values (Tab. 5). Differences were significant ($P \leq 0.05$) for both groups. While talking about meat : fat ratio homozygotes GC showed significantly lower values than heterozygotes ($P \leq 0.05$).

Table 5

Least squares means (LSM) and their standard errors (SE) for bulls' traits affected significantly by DGAT-1 genotypes

	Genotype					
	AA:AA		AA:GC		GC:GC	
Carcass traits	LSM	SE	LSM	SE	LSM	SE
Content fat in valuable cuts, kg	5.35	0.19	5.10^{a}	0.15	5.39^{a}	0.16
Valuable cuts of carcass-side, %	60.37^{ab}	0.33	61.12^{a}	0.26	61.22^{b}	0.27
Fat of carcass-side, %	9.88	0.36	9.45^{a}	0.28	10.03^{a}	0.29
Meat fat ratio	7.99	0.3	8.20^{a}	0.23	7.74^{a}	0.24

[aa] – Within rows means bearing the same subscripts differ significantly at $P \leq 0.05$.

For technological traits significant differences were found only for water holding capacity (Tab. 6). Heterozygotes exhibit maximum values of the studied trait (29.81 g/cm 2) and differed significantly from AA homozygotes ($P \leq 0.05$). Sakowski et al.(2000) investigated few cattle breeds and cross breeds and obtained similar results. The Angus breed had the highest water holding capacity (33.2 g/cm 2).

Table 6

Least squares means (LSM) and their standard errors (SE) for bulls' traits affected significantly by DGAT-1 genotypes

Physicochemical and technological parameters	Genotype					
	AA:AA		AA:GC		GC:GC	
	LSM	SE	LSM	SE	LSM	SE
Water holding capacity, cm^2/g	26.02[a]	1.58	29.81[a]	1.5	28.74	1.32

[aa] Within rows means bearing the same subscripts differ significantly at $P \leq 0.05$.

A fatty acids profile in the C12 – C20_5 range, including CLA was investigated (Tab. 7). The bulls of GC:GC *DGAT-1* genotype presented significantly higher values for lauric acid ($P \leq 0.05$) than AA:GC heterozygotes (+0.011). Heterozygotes presented higher content of CLA than AA:AA inividuals. This results are not in agreement with results obtained for dairy cattle, where lysine variant (AA) is connected to the higher fat content in milk (Winter et al. 2002; Gisart et al. 2002). According to Moore *et al. (2003)*, there is no association between the *DGAT-1* polymorphism and fat content. However, due to the low number of homozygotes these results may not be statistically reliable, especially in concern to the research made by Thaller et al. (2003b) on Holstein and Charolaise breeds.

Table 7

Least squares means (LSM) and their standard errors (SE) for bulls' traits affected significantly by DGAT-1 genotypes

Fatty acid	Genotype					
	AA:AA		AA:GC		GC:GC	
	LSM	SE	LSM	SE	LSM	SE
C12	0.018	0.007	0.014[a]	0.006	0.025[a]	0.007
CLA	0.07[a]	0.01	0.10[a]	0.01	0.09	0.01

[aa] Within rows means bearing the same subscripts differ significantly at: small letters – $P \leq 0.05$

Molecular studies have shown, that nonconservative lysine / alanine (K232A) dinucleotide substitution (in positions 10,433 and 10,434) strongly influenced milk and fat yield (Grisart et al. 2002; Winter et al. 2002; Thaller et al. 2003a) and fat content in beef cattle (Moore et al. 2003; Thaller et al. 2003b; Casas et al. 2005). In particular, the lysine encoding allele (allele K) is associated with increased fat content of milk compared to the alanine aminoacid residue (allele A) (Winter et al. 2002; Thaller et al. 2003a). Moreover, it was shown that the *K* allele is related to an increase of saturated and a decrease of unsaturated fatty acids share in milk (Schennink et al. 2007;. Grisart et al. 2004) conducted expression studies and showed that two variants of *DGAT-1* gene had a different mRNA expression level, which is due to the mutation. The lysine variant was characterized by the higher enzyme activity level (measured as V_{max}) and higher triglyceride produce (Grisart et al. 2004). Kaupe and coworkers assessed allele frequency for 38 breeds of *Bos taurus* and *Bos indcus*. The alanine variant, which is connected to higher milk yield, occurs more frequently in dairy breeds due to selection. These results are in contrast with beef cattle breeds (Belgian Blue, Hereford, Piemontese) where A allele exhibits low frequency (Kaupe et al. 2004).

4. CONCLUSION

Results obtained so far suggest tha2//2/.t the DGAT-1 gene is a better marker for milk performance traits in dairy cattle than for meat quality traits.

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